Rapid, quantitative adipose conversion of chicken fibroblasts by high concentrations of chicken serum or plasma[1]

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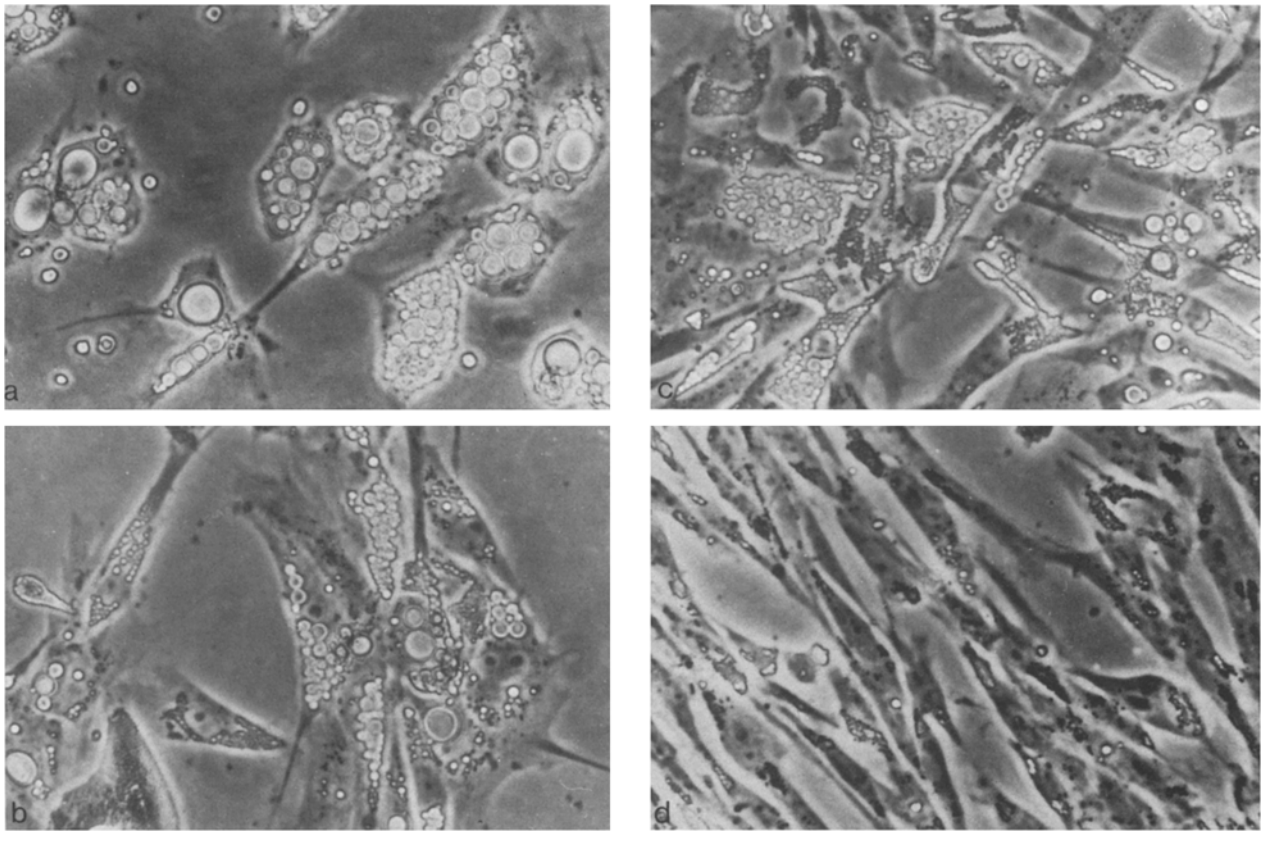
Summary. Rapid, quantitative adipose conversion of chicken fibroblasts occurs when these cells are cultured in undiluted commercial chicken serum or plasma. Fresh serum and plasma acquire this property after ageing.

Spontaneous adipose conversion has been observed in selected sublines of mouse 3T3 cells[3–6]. Adipose conversion of 3T3 sublines occurs after attainment of cell confluency, takes several weeks to develop and affects only a small proportion of cells in a culture. The conversion is more pronounced in 30% than in 10% serum and is influenced by lipotrophic and lipolytic hormones[5,7,8]. Adipose conversion of 3T3 cells has been reported to be prevented by bromodeoxyuridine, a piece of evidence which has been taken to indicate that the change represents differentiation, rather than lipid degeneration[4].

We now report the rapid, quantitative adipose conversion, by undiluted commercial chicken serum or plasma, of early passage cultures of unselected chicken fibroblasts. Quarternary cultures of fibroblasts were prepared, by our published method[9–11], from the pectoral muscles of 8-week-old male chickens. The complete medium used for the growth of stock cultures and the seeding of experimental cultures consisted of 95 parts of our synthetic medium[11] and 5 parts of heat defibrinogenated (56 °C × 30 min) chicken plasma, this latter reagent developed in our laboratory[9,10]. All cultures were maintained in a 95% air/5% CO_2 incubator at chicken body temperature (41.9 °C). Experimental cultures were seeded at 60,000 cells per 35-mm culture dish. On the day following seeding, experimental cultures were changed to test media consisting of undiluted serum or plasma and serum or plasma diluted to 50%, 30% and 5% with synthetic medium.

After 2 days of culture in undiluted, heat-inactivated (56 °C × 30 min) commercial chicken serum (GIBCO, Grand Island, New York, and Colorado Serum Co, Denver), proliferation had ceased and virtually all cells showed massive accumulation of large, refractile cytoplasmic inclusions (figure, a). These inclusions were Oil Red O-positive and stained intensely black with osmic acid, indicating that they were lipid. In some of the cells single, very large lipid droplets occupied a central position, as they do in mature adipocytes. These large, central lipid droplets appeared to form by coalescence of smaller droplets. The formation of lipid droplets was accompanied by increase in cell size and retraction of cell processes, as has been reported in the 3T3 system.

After more than 2 days of culture in undiluted serum or plasma (replenished every 48 h), further change in the cells was limited to progressive coalescence of smaller lipid droplets and enlargement of the larger, central droplets. The adipose change was not reversed when the undiluted serum was replaced with growth medium (95% synthetic



Appearance of chicken fibroblasts after 2 days of culture in the following concentrations of commercial chicken serum: *a* Undiluted, *b* 50%, *c* 30%, *d* 5%. × 130, phase contrast.

medium/5% defibrinogenated plasma); the adipose cells, however, remained viable during a number of days of observation in the latter medium.

A gradient of decreasing adipose conversion and increasing cell proliferation was observed in cultures containing, respectively, 50%, 30% and 5% commercial serum (figure, b–d). In this last test medium, little adipose conversion and maximal cell proliferation were observed. Identical adipose changes were observed when commercial chicken plasma (heparinized) was used, over the same range of concentrations (undiluted, 50%, 30% and 5%), in place of commercial chicken serum. Rapid, quantitative adipose conversion was also observed when Rous sarcoma virus-infected chicken fibroblasts were cultured in undiluted commercial serum or plasma.

It was puzzling to observe that adipose conversion of fibroblasts occurred in the presence of high concentrations of commercial chicken serum or commercial plasma but occurred little, if at all, in fresh serum or heparinized or heat-defibrinogenated plasma prepared in our own laboratory. (The commercial and fresh sera did not differ, significantly, in their content of triglycerides or cholesterol). This matter was resolved when we observed that serum and plasma prepared in our own laboratory, and then allowed to age at 41.9 °C for 2 weeks, would induce adipose conversion as does commercial serum or plasma.

Adipose conversion occurred when commercial chicken serum was combined with an equal part of fresh serum indicating that the commercial serum contained an initiator, rather than lacked an inhibitor, of the change. Adipose conversion did not occur when an ultrafiltrate of commercial serum (20,000 molecular weight cutoff) was combined with an equal part of fresh serum, indicating that the active principle in commercial serum is a macromolecule or is bound to a macromolecule.

Adipose conversion of chicken fibroblasts by serum or plasma, as described here, appears to be very similar, if not identical, to the spontaneous adipose conversion that has been described with sublines of 3T3 cells. Our results indicate that chicken fibroblasts, like some 3T3 sublines, are susceptible to this change. Unlike the 3T3 systems, however, adipose conversion of chicken fibroblasts is rapid (2 days) and quantitative and does not require cell confluency. Indeed, adipose conversion of chicken fibroblasts by undiluted chicken serum or plasma causes cell proliferation to cease at low (subconfluent) culture densities.

Adipose conversion in the 3T3 systems, as noted earlier, is inhibited by bromodeoxyuridine at a concentration of 5×10^{-6} M. Adipose conversion of chicken fibroblasts was not, on the other hand, affected when these cells were allowed to divide 5 times in growth medium containing 10^{-5} M bromodeoxyuridine, before being passaged in the same and then exposed to undiluted serum containing the above concentration of the drug. It is possible that the rapidity of the adipose change in the chicken system precludes an inhibitory effect of bromodeoxyuridine. On the other hand, it is possible that the adipose change that we describe here represents a form of lipid degeneration, rather than representing true adipocyte differentiation. In either case, we have demonstrated that the ageing of serum or plasma generates a principle that is capable of causing adipose change in cultured fibroblasts. Further study of this principle and of the change that it causes may provide worthwhile information regarding control of cellular differentiation or the initiation of a specific form of cellular damage.

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An actin-destabilizing factor is present in human plasma[1]

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Summary. Plasma and serum of humans or experimental animals contain a factor which destabilizes F-actin. The factor has no DNAse or thrombin activity and after incubation with F-actin does not modify the position of the actin band on a SDS polyacrylamide gel. Hence it probably depolymerizes F-actin.

Human anti-actin autoantibodies (AAA) have been used in several laboratories as a tool for the study of actin distribution in cells under different conditions[2–5]. Preliminary work from this laboratory had indicated that there is a difference in the pattern and intensity of AAA stainings after using whole sera or affinity column purified antibodies. Here, we report that the plasma and serum of humans or experimental animals (e.g. rabbit, rat, guinea-pig) contain an actin-destabilizing factor responsible for this staining difference.

We used the sera of 2 patients with chronic aggressive hepatitis having a titer of 1/1280 and 1/640 respectively when tested on rat intestinal smooth muscle. The sera were passed on column of CNBr activated sepharose[6] or on a glutaraldehyde immunoabsorbent[7] covalently linked with rabbit skeletal muscle actin[8], followed by elution of the antibody at pH 2.7. The specificity of these antibodies was tested by immunodiffusion, immunoelectrophoresis and immunofluorescence as described previously[9,10]. Mouse fibroblast cultures were prepared from 12–14-day-old embryos of Swiss albino CR-1 mice[11]. Secondary cultures were always used. The cultures were fixed and stained when non-confluent and at confluence. We examined also frozen sections (4 μm thick) of normal rat liver. For immunofluorescent staining, cells on glass coverslips were fixed 30 sec